PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 April 2001 (03.04.01)	
International application No. PCT/IL99/00386	**Applicant's or agent's file reference** 117025.7 DB
International filing date (day/month/year) 14 July 1999 (14.07.99)	**Priority date** (day/month/year)
Applicant PRIMOR, Naftali et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

11 February 2001 (11.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PA NT COOPERATION TREAT

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel Aviv
ISRAËL

Date of mailing (day/month/year) 14 January 2002 (14.01.02)	
Applicant's or agent's file reference 117025.7 DB	**IMPORTANT NOTIFICATION**
International application No. PCT/IL99/00386	**International filing date** (day/month/year) 14 July 1999 (14.07.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
SHULOV INSTITUTE FOR VENOM RESEARCH LTD. Prof. Bergman Street 2/224 76705 Rehovot Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
S.I.S. SHULOV INSTITUTE SCIENCE LTD. Prof. Bergman Street 2/224 76705 Rehovot Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Beate GIFFO-SCHMITT
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US)

PCT/IL99/00386

PATENT COOPERATION TREATY

L
10/030380
5 C C O

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel Aviv
ISRAËL

Date of mailing (day/month/year) 06 février 2002 (06.02.02)	
Applicant's or agent's file reference 117025.7 DB	**IMPORTANT NOTIFICATION**
International application No. PCT/IL99/00386	**International filing date** (day/month/year) 14 juillet 1999 (14.07.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
S.I.S. SHULOV INSTITUTE SCIENCE LTD. Prof. Bergman Street 2/224 76705 Rehovot Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
S.I.S. SHULOV INSTITUTE FOR SCIENCE LTD. Oppenheimer Street 10 Park Tamar 76701 Rehovot Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Anne KARKACHI
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004641885

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
117025.7 DB		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 99/00386	14/07/1999	
Applicant		
SHULOV INSTITUTE FOR VENOM RESEARCH LTD. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 99/00386

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 15 and 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/ 99/00386

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/58 A61P29/02 C07K2/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E,L	WO 99 36078 A (PRIMOR NAFTALI ;SHULOV BARKAN SHLOMIT HF (IL); SHULOV INST FOR VEN) 22 July 1999 (1999-07-22) Cf. priority document IL-A-123001 the whole document	1-21
X	& IL 123 001 A (SHULOV INST FOR VENOM RESEARCH) 6 December 1998 (1998-12-06) ---	1-21
A	EP 0 246 861 A (HERNANDEZ PLATA GUILLERMO JOSE ;COSTA LUIS ALBERTO (AR); CONI MOLI) 25 November 1987 (1987-11-25) example; claims --- -/--	1-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2000	20/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Teyssier, B

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna[redacted]l Application No
PCT/[redacted] 99/00386

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MANCIN A C ET AL: "The analgesic activity of crotamine, a neurotoxin from Crotalus durissus terrificus (South American rattlesnake) venom: A biochemical and pharmacological study." TOXICON, vol. 36, no. 12, December 1998 (1998-12), pages 1927-1937, XP000892432 the whole document	1-21

3

INTERNATIONAL SEARCH REPORT

Information [redacted] patent family members

Internat[redacted] Application No
PCT/[redacted] 99/00386

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9936078 A	22-07-1999	IL 123001 A	06-12-1998
		AU 1888799 A	02-08-1999
EP 0246861 A	25-11-1987	AT 86863 T	15-04-1993
		DE 3784768 A	22-04-1993
		ES 2054668 T	16-08-1994
		JP 1997077 C	08-12-1995
		JP 7020875 B	08-03-1995
		JP 63033334 A	13-02-1988

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

REINHOLD COHN AND PARTNERS
P.O.B. 4060
61040 Tel Aviv
ISRAEL

PCT

WRITTEN OPINION

(PCT Rule 66)

ND 5.07 2001 01:17

Date of mailing *(day/month/year)*	05.04.2001
Applicant's or agent's file reference: 117025.7 DB	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00386	14/07/1999	14/07/1999

International Patent Classification (IPC) or both national classification and IPC

A61K35/58

Applicant

SHULOV INSTITUTE FOR VENOM RESEARCH LTD. et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 14/11/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Böhmerova, Eva 7859

Formalities officer (incl. extension of time limits)
Hundt, D
Telephone No. +49 89 2399 8042

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-17 as originally filed

Claims, No.:

1-21 as originally filed

Drawings, sheets:

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 15, 16,

because:

   ☒ the said international application, or the said claims Nos. 15, 16 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1 - 3, 8 - 11, 13 -15, 17: No
Inventive step (IS)	Claims	4 - 7, 12, 16, 18 - 21: No

Industrial applicability (IA) Claims

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: EP-A-0 246 861 (HERNANDEZ PLATA GUILLERMO JOSE; COSTA LUIS ALBERTO (AR); CONI MOLI) 25 November 1987 (1987-11-25)

D2: MANCIN A C ET AL: 'The analgesic activity of crotamine, a neurotoxin from Crotalus durissus terrificus (South American rattlesnake) venom: A biochemical and pharmacological study.' TOXICON, vol. 36, no. 12, December 1998 (1998-12), pages 1927-1937, XP000892432

Re Item III

Non-establishment of opinion with regard to industrial applicability

Claims 15, 16 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

i) Novelty

D1 (abstract) discloses crotoxine complex sub-units A and B isolated from the crude venom of *Crotalus Durissus Terrificus* and the use thereof for treating pain. The crotoxine complex was proven to be non-toxic (page 12, line 37). This disclosure destroys novelty of claims 1 to 3 and 8 and 9. The product-by-process features present in claims 1 and 2 do not prove that the claimed product differs from the product known from **D1**.

In **D1**, the crotoxine complex subunits were prepared by applying the venom to an ion-exchange column and eluting the fraction with an aqueous buffer (see Example), which

anticipates novelty of claim 17.

D1 further discloses a pharmaceutical composition for use as analgesic comprising the crotoxine complex subunits A and B (page 3, line 37-39) administered by intramuscular or sub-cutaneous injection (page 10, line 40), which anticipates novelty of claims 11, 13 and 14.

D1 further claims the use of crotoxine complex sub-units A and B for the manufacture of a medicament for treating pain (claim 9), which anticipates novelty of claim 10.

D2 (abstract) discloses the analgesic activity of crotamine, a protein from *Crotalus durissus terrificus* venom. Crotamine was proven to be substantially non-toxic in the dosage possessing high analgesic effect (page 1935, line 10 - 15). Therefore, the disclosure of **D2** anticipates novelty of claims 1 to 3. The product-by-process features present in claims 1 and 2 do not prove that the claimed product differs from the product known from **D2**.

Therefore, claims 1 to 3, 8 to 11, 13, 14, 15 and 17 are no allowable under Art. 33(2) PCT.

ii) Inventive step

The only new feature over **D1** set out in dependent claims 4 to 7 is the use of the venom from different snake species. As the analgesic effect of snake venom is known since antiquity (see **D1**, page 2, line 34), the use of a different snake species as a source of the venom is obvious for the person skilled in the art.

The only new feature over **D1** set out in dependent claims 12 and 16 is a topical administration of the pharmaceutical composition comprising said fraction from snake venom. However, as Viprosalum - an ointment comprising snake venom, is known in the art (see description of the application, page 4, line 6 - 9), the topical administration of the said fraction represents an alternative obvious to the person skilled in the art.

The only new feature over **D1** set out in dependent claims 18 to 21 are the different conditions of the ion-exchange chromatography. However, the use of different column

and elution buffers for ion-exchange chromatography is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill.

Therefore, the subject-matter of claims 4 to 7, 12, 16 and 18 to 21 does not involve an inventive step according to Art. 33(3) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 36078 A	22.07.99	13.01.99	20.01.98

This document appears to be particularly relevant as regard the subject-matter of the present application and as a consequence thereof it may be opposed to the present application in its national or regional phases.

Re Item VIII
Certain observations on the international application

The use of trademarks and similar expressions in the claims makes the claims unclear as it may not be guaranteed that the product referred to is not modified while maintaining its name during the term of the patent and therefore, the trademarks and similar expressions should be removed from the claims (PCT International Preliminary Examination Guidelines, Chapter III-4.5b).

The term "Mono Q ion-exchange chromatography" represents a expression of the above kind and the presence thereof makes claims 1, 2 and 18, as well as the claims dependent thereon, unclear according to Art. 6 PCT.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
REINHOLD COHN AND PARTNERS
P.O.B. 4060
61040 Tel Aviv
ISRAEL

RECEIVED
29-07-2001

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	23.07.2001
Applicant's or agent's file reference: 117025.7 DB	**REPLY DUE** **within 2 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00386	14/07/1999	14/07/1999

International Patent Classification (IPC) or both national classification and IPC
A61K35/58

Applicant
SHULOV INSTITUTE FOR VENOM RESEARCH LTD. et al.

1. This written opinion is the **second** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 14/11/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
0049-89-2399-7859
Böhmerova, EVA

Formalities officer (incl. extension of time limits)
Hundt, D
Telephone No. +49 89 2399 8042

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-17	as originally filed				

Claims, No.:

2-9,11-21	as originally filed				
1,10	as received on	24/06/2001	with letter of	24/06/2001	

Drawings, sheets:

1/6-6/6	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 15, 16,

because:

☒ the said international application, or the said claims Nos. 15, 16 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement
Novelty (N) Claims 1 - 5, 8 -21: No

Inventive step (IS)	Claims	6, 7: No
Industrial applicability (IA)	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: EP-A-0 246 861 (HERNANDEZ PLATA GUILLERMO JOSE; COSTA LUIS ALBERTO (AR); CONI MOLI) 25 November 1987 (1987-11-25)

D2: MANCIN A C ET AL: 'The analgesic activity of crotamine, a neurotoxin from Crotalus durissus terrificus (South American rattlesnake) venom: A biochemical and pharmacological study.' TOXICON, vol. 36, no. 12, December 1998 (1998-12), pages 1927-1937, XP000892432

During the further examination of the present application, the additional document cited in the International Search Report has been found as being relevant:

D3: IL123001 (PRIMOR N, SHULOV A D, SHULOV A) 6 December 1998 (1998-12-06)

The full text of **D3** is not in the Examiner's disposal, however as **D3** is a family member of PCT application WO 99/36078, the Examiner assumes that the content of **D3** is identical with the content of WO 99/36078 as published. Therefore, the following references to the relevant passages concern the text of WO 9936078 as published. If the Applicant does not agree with the above assumption he is asked to provide an English translation of **D3**.

Re Item III

Non-establishment of opinion with regard to industrial applicability

Claims 15, 16 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

A) Objections based on **D3**

D3 (Examples) discloses purification of Zephalin, a substantially non-toxic fraction from *Vipera xanthina palestinae* venom having the analgesic affect. The preferred isolation method using a Mono Q column (Pharmacia) (page 8, second paragraph) is identical with the isolation method according to the present invention. The characteristics of Zephalin (see examples IIB, IIC, III, IV, figure 2A, 2B) are identical with the characteristics of the fraction according to the present invention. Zephalin is used for topical treatment of pain (Example IVB, Table 3,4) with the same results as a fraction of the present invention. **D3** (claims) discloses as well a product/derivative of Zephalin and a pharmaceutical composition for treatment of pain comprising Zephalin for topical or parenteral administration.

The above disclosure of **D3** anticipates novelty of claims 1 to 5 and 8 to 21 and therefore claims 1 to 5 and 8 to 21 are not allowable under Art. 33(2) PCT.

The only new feature over **D3** set out in dependent claims 6 and 7 is the fact that the fraction is isolated from the venom of different snake species. The problem to be solved as defined in the description is to provide an analgesic substance isolated form snake venom which is substantially non-toxic. Taking into account teaching of **D3**, which is considered as the closest prior art, the problem can be re-defined as to provide an alternative to Zephalin - an analgesic non-toxic substance isolated form *Vipera xanthina palestinae* venom. However as the analgesic effect of snake venom is known since antiquity (see **D1**, page 2, line 34), the use different snake species' venom in the identical purification process to obtain analgesic non-toxic fraction thereof appears to be obvious for the person skilled in the art. There appears to be no prove on file that it was not obvious to use the snake venom of *Crotalus adamanteus* and *Naja melanoleuca* as an alternative to the venom of *Vipera palestinae*, e.g. that it was not possible to predict that by applying the same purification method as disclosed in **D3** to the venom of these two species, a non-toxic analgesic fraction can be obtained.

Therefore, claims 6 a and 7 are not allowable under Art. 33(3) PCT.

B) Comments on the Applicant's response

B1) Amendments

With the Applicant's letter of June 24, 2001, claims 1 and 10 have been amended by defining the analgesic effect of the fraction as occurring "after a lag period". However, the term "lag period" is undefined and unclear, there is not exact definition of the lag period after which the fraction is active in the description. Moreover, existence of a lag period before the occurrence of the effect is a typical characteristic of all analgesics. Therefore, "lag period" cannot be recognised as a distinctive product feature.

The Applicant is to be informed that after omitting all non-distinctive features from the main claim (e.g. "lag period" and the process features), the only relevant product features of the claimed fraction are:
- substantially non-toxic,
- isolated from snake venom, and
- having an analgesic effect.

B2) Arguments concerning document **D1**

The fact that **D1** does not provide any data to support the claimed analgesic effect and non-toxicity of the crotoxine complex subunits A and B does not play any role in considering the relevance thereof for assessing novelty and inventiveness of the present application. **D1** discloses a crotoxine complex sub-units A and B as a non-toxic analgesic fraction isolated form snake venom by ion-exchange chromatography, which definition clearly falls into the scope of the present claim 1. The fact, that the present application discloses data proving non-toxicity and analgesic effect of the claimed product while **D1** does not, cannot establish novelty of the anticipated claims over **D1**. Therefore, claims 1, 2, 3, 8 to 11, 13, 14 and 17 are still considered as being not novel over **D1** and therefore not allowable under Art. 33(3) PCT.

B3) Arguments concerning document **D2**

As the fact of acting after the lag period cannot be considered as a distinctive **product** feature (see B1), this feature cannot establish novelty of claims 1 to 3 over **D3** disclosing crotamine as an substantially non-toxic fraction having analgesic effect and being obtained from snake venom. Therefore, claims 1 to 3 are still considered as

being not novel over **D2** and therefore not allowable under Art. 33(3) PCT.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 36078 A	22.07.99	13.01.99	20.01.98

This document appears to be particularly relevant as regard the subject-matter of the present application and as a consequence thereof it may be opposed to the present application in its national or regional phases.

Re Item VIII

Certain observations on the international application

The use of trademarks and similar expressions in the claims makes the claims unclear as it may not be guaranteed that the product referred to is not modified while maintaining its name during the term of the patent and therefore, the trademarks and similar expressions should be removed from the claims (PCT International Preliminary Examination Guidelines, Chapter III-4.5b).

The term "Mono Q ion-exchange chromatography" represents a expression of the above kind and the presence thereof makes claims 1, 2 and 18, as well as the claims dependent thereon, unclear according to Art. 6 PCT.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

REINHOLD COHN AND PARTNERS
P.O.B. 4060
61040 Tel Aviv
ISRAEL

RECEIVED

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	09.10.2001
Applicant's or agent's file reference 117025.7 DB	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00386	14/07/1999	14/07/1999

Applicant
SHULOV INSTITUTE FOR VENOM RESEARCH LTD. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hundt, D Tel.+49 89 2399-8042

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
117025.7 DB		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00386	14/07/1999	14/07/1999
International Patent Classification (IPC) or national classification and IPC A61K35/58		
Applicant SHULOV INSTITUTE FOR VENOM RESEARCH LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/02/2001	09.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Böhmerova, E Telephone No. +49 89 2399 7859

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00386

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-17	as originally filed

Claims, No.:

1-18	as received on	20/09/2001	with letter of	20/09/2001

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 12, 13.

because:

☒ the said international application, or the said claims Nos. 12, 13 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-18

	No:	Claims	-
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-18
Industrial applicability (IA)	Yes:	Claims	1-11, 14-18
	No:	Claims	-

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following document:

D3: IL123001 (PRIMOR N, SHULOV A D, SHULOV A) 6 December 1998 (1998-12-06)

The full text of **D3** is not in the Examiner's disposal, however as **D3** is a family member of PCT application WO 99/36078, the Examiner assumes that the content of **D3** is identical with the content of WO 99/36078 as published. Therefore, the following references to the relevant passages concern the text of WO 9936078 as published.

Re Item III

Non-establishment of opinion with regard to industrial applicability

Claims 12, 13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Disclosure of D3

D3 (Examples) discloses purification of Zephalin, a substantially non-toxic fraction from *Vipera xanthina palestinae* venom having the analgesic affect. The preferred isolation method using a Mono Q column (Pharmacia) (page 8, second paragraph) is identical with the isolation method according to the present invention. The characteristics of Zephalin (see examples IIB, IIC, III, IV, figure 2A, 2B) are identical with the characteristics of the fraction according to the present invention. Zephalin is used for topical treatment of pain (Example IVB, Table 3,4) with the same results as a fraction of the present invention. **D3** (claims) discloses as well a product/derivative of Zephalin and a pharmaceutical composition for treatment of pain comprising Zephalin for topical or parenteral administration.

Novelty

Claims 3 and 4 are considered as being novel as none of the prior art documents discloses a fraction obtained from the species *Crotalus adamanteus* or *Naja melanoleuca.* The subject-matter of new claims 1, 2 and 5 to 18 has been made novel over the disclosure of **D3** by disclaiming the fraction obtained from the snake specie *Vipera xanthina.* Therefore, the present claims as they state are considered as being novel under Art. 33(2) PCT.

However, it must be expected that in certain Contracting States, especially in the International Authority in charge of establishing this International Preliminary Examination Report, the novelty of claims 1, 2 and 5 to 18 might not be acknowledged for the following reasons:

(i) The use the disclaimer technique is acceptable only in the cases where another definition by positive means is not possible (see PCT Guidelines Section IV, III-4-12),
(ii) the disclaimer is allowable only where the disclosure is accidental in that it has no real relationship with the problem the invention aims at solving, and
(iii) the disclaimer is not allowed when the disclaimed subject-matter is to be found in a document which can be used for the purpose of assessing the inventive step of the claim where the disclaimer is to be found.

As in the present case document **D3** does not represent an accidental disclosure, moreover it is considered as the closest prior art in assessing inventive step of the present claims, the disclaimer in question might not be accepted in some Contracting States. It might be considered as added matter having no support in the application as originally filed which violates Article 19 PCT. Consequently, the examination would be performed on the present set of the claims as if no disclaimer were present. In such a case, the disclosure of **D3** would be novelty destroying for the claims 1, 2 and 5 to 18.

Inventive step

The only new feature over **D3** set out in present set of the claims is the fact that the fraction is isolated from the venom of different snake species. The problem to be solved as defined in the description is to provide an analgesic substance which is substantially

non-toxic isolated form snake venom. Taking into account teaching of **D3**, which is considered as the closest prior art, the problem can be re-defined as to provide an alternative to Zephalin - an analgesic non-toxic substance isolated form *Vipera xanthina palestinae* venom. However, the use different snake species' venom in the identical purification process to obtain analgesic non-toxic fraction thereof appears to be obvious for the person skilled in the art. There appears to be no proof on file that it was not obvious to use the snake venom of other snake species as an alternative to the venom of *Vipera xanthina palestinae*, e.g. that it was not possible to predict that by applying the same purification method as disclosed in **D3** to the venom of these two species, a non-toxic analgesic fraction can be obtained. Moreover, the experimental data present in the application shows that the fractions obtained from *Vipera russelli* and *Crotalus adamanteus* have weaker analgesic effect than the fraction known from **D3** (see examples VI and VII). No results showing the analgesic activity of the fraction obtained from the venom of *Naja melanoleuca* are present (Example VIII).

Therefore, claims 1 to 18 are not allowable under Art. 33(3) PCT.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 36078 A	22.07.99	13.01.99	20.01.98

This document appears to be particularly relevant as regard the subject-matter of the present application and as a consequence thereof it may be opposed to the present application in its national or regional phases.

Re Item VIII

Certain observations on the international application

The use of trademarks and similar expressions in the claims makes the claims unclear

as it may not be guaranteed that the product referred to is not modified while maintaining its name during the term of the patent and therefore, the trademarks and similar expressions should be removed from the claims (PCT International Preliminary Examination Guidelines, Chapter III-4.5b).

The term "Mono Q ion-exchange chromatography" represents a expression of the above kind and the presence thereof makes claims 1, 2 and 15, as well as the claims dependent thereon, unclear according to Art. 6 PCT.

10/030380
JC13 Rec'd PCT/PTO 10 JAN 2002

CLAIMS:

1. A substantially non-toxic fraction isolated from snake venom having the characteristics of a fraction purified from said venom by Mono Q ion-exchange chromatography, wherein said fraction has an analgesic effect after a lag period, and wherein said snake is selected from the group of snake families consisting of *Atractaspidae, Elapidae, Crotolidae, Hydrophidae* and *Viperidae*, with the exception of *Vipera xanthina*.

2. A fraction according to Claim 1 wherein said chromotography is carried out on a Mono Q column in 20mM Tris-HCl buffer pH 7.0, and the fraction elutes at 12-28 minutes.

3. A fraction according to Claim 1 wherein said *Crotolidae* is *Crotalus adamanteus*.

4. A fraction according to Claim 1 wherein said *Elapidae* is *Naja melanoleuca*.

5. A product obtained from the fraction of Claim 1 which retains said properties of the fraction.

6. A derivative of the product of Claim 5 which retains said properties of the fraction of Claim 1.

7. Use of a fraction according to Claim 1 in the preparation of a pharmaceutical composition for use as an analgesic acting after a lag period.

8. A pharmaceutical composition for use as an analgesic comprising a substantially non-toxic fraction according to Claim 1 and a pharmaceutically acceptable carrier or excipient.

9. A pharmaceutical composition according to Claim 8 for topical administration.

10. A pharmaceutical composition according to Claim 8 for parenteral administration.

11. A pharmaceutical composition according to Claim 8 for the treatment of pain.

12. A method for the relief of pain of a subject comprising administrating to said subject a substantially non-toxic fraction according to Claim 1.

13. A method according to Claim 12 wherein said fraction is topically administered.

14. A method for isolating a substantially non-toxic fraction from snake venom, wherein said fraction has an analgesic effect, comprising applying whole venom to an ion exchange column and eluting the fraction with an aqueous buffer, wherein said snake is selected from the group of snake families consisting of *Atractaspidae, Elapidae, Crotolidae, Hydrophidae* and *Viperidae*, with the exception of *Vipera xanthina*.

15. A method according to Claim 14 wherein said column is a Mono Q ion-exchange column.

16. A method according to Claim 15 wherein said column is eluted with a Tris-HCl buffer or with an ammonium acetate buffer.

17. A method according to Claim 16 wherein the concentration of said buffer is 20mM and the pH is in the range of 6.8-7.5.

18. A method according to Claim 17 wherein said fraction elutes at 12-28 minutes.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

REINHOLD COHN AND PARTNERS
P.O.B. 4060
61040 Tel Aviv
ISRAEL

PCT

NOTIFICATION CONCERNING INFORMAL COMMUNICATIONS WITH THE APPLICANT

(PCT Rule 66.6)

	Date of mailing *(day/month/year)* 13.09.2001
Applicant's or agent's file reference 117025.7 DB	**TRANSMITTAL FOR INFORMATION**
International application no. PCT/IL99/00386	International filing date *(day/month/year)* 14/07/1999
Applicant SHULOV INSTITUTE FOR VENOM RESEARCH LTD. et al.	

An informal communication took place on 06/09/2001, between the International Preliminary Examining Authority and the applicant / the agent.

A copy of the note on that communication (Form PCT/IPEA/428) is herewith transmitted for your information.

Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hundt, D Telephone No. +49 89 2399-8042

Form PCT/IPEA/429 (july 1993)

Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens
Patent Cooperation Treaty
Traîté de coopération en matière de brevets

PCT

Application No.: PCT/IL99/00386

Note on an informal communication by telephone with the Applicant

A copy of this note is being sent to the Applicant for information

Participants

Agent: Patinkin J.

Examiner(s): Böhmerova, E

Summary of the communication

The problem of novelty of the present set of the claims over document D3 (IL123001) has been discussed.

The Representative proposed to file a new set of the claims with the disclaimer excluding the subject-matter disclosed in D3, concretely the snake venom fraction obtained from the *Vipera xanthina palestinae,* in order to establish novelty over D3.

The Examiner took position on the above proposal as follows:
It is correct that there is no legal base in the PCT or PCT Guidelines which would allow to refuse establishing novelty by a disclaimer. Therefore, amended claims with the proposed disclaimer are basically acceptable. However, the Authority in charge of establishing the International Preliminary Examination Report, in the absence of any guidance in the PCT Guidelines with respect to the use of disclaimers in cases where the disclaimed disclosure is not accidental, is not obliged to take into account the possible differences of practice which might exist in all Contracting States in this respect and of which it might possibly not be aware of.

It seems, however, to be a generally and widely spread practice (i) to use the disclaimer technique only in the cases where another definition by positive means is not possible (see PCT Guidelines Section IV, III-4-12), (ii) not to allow disclaimer where the disclosure is not accidental in that it has no real relationship with the problem the invention aims at solving and (iii) not to allow disclaimers as well when the disclaimed subject-matter is to be found in a document which is the closest prior art for the purpose of assessing the inventive step of the claim where the disclaimer is to be found.

Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens
Patent Cooperation Treaty
Traîté de coopération en matière de brevets

PCT

Application No.: PCT/IL99/00386

Therefore should a new set of claims be filed with the proposed disclaimer, then it might be that IPER will be negative, at least for certain Contracting States.

06/09/2001
.....
Date *(day / month / year)*

Böhmerova, E
.....
Authorized officer of IPEA

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1899-1973

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EUROPEAN PATENT OFFICE
D-80298 Munich
GERMANY

Attention: E. Bohmerova

Dear Sirs,

Re: Patent Application No. PCT/IL99/00386
"Analgesic from snake venom"
in the name of Shulov Institute For Venom Research, Ltd.
Our Ref: 117025-7

This is in response to the Written Opinion of April 5, 2001, in accordance with Rule 66.3.

We enclose a replacement page 18 in triplicate and a marked-up copy of the original page 18 indicating the amendments.

Claims 1 and 10 have been amended.

The Claims have been amended by defining the analgesic effect as occurring "*after a lag period*". Support for this amendment may be found in the specification on page 6, lines 15-18, and on page 7, lines 25-31, from which it may be seen that the active fraction is administered at least one day *before* induction of pain.

The Examiner cites document D1 as destroying the novelty and inventiveness of the invention. Applicant respectfully disagrees for the following reasons:

1. Although D1 states that crotoxine exhibits a large analgesic effect (pg. 12, line 56), it brings absolutely no data to support this proposition. This is a mere unsupported claim of the authors, and cannot be used to contradict the novelty of the fraction of the invention, whose analgesic effect is supported by experimental evidence (pg. 10, lines 7-25; pg. 15, lines 17-22; pg. 16, lines 10-15).

2. Furthermore, D1 states (pg. 13, lines 10-13) that patients frequently feel pain after administration of the drug.

3. Although the analgesic effect of snake venom is claimed by D1 to be known since antiquity, this is true only of crude or raw venom (pg. 2, lines 34-41). However, no one before the priority date had characterized a specific fraction of snake venom having the properties of the fraction of the invention.

4. D1 further states that no toxicity effects are produced (pg. 12, line 37). Once again, absolutely no data is brought to support this proposition, as opposed to the fraction of the invention, whose non-toxicity is experimentally proved (pgs 11-15, pg. 16, lines 16-19; pg. 17, lines 1-4).

5. Furthermore, crotoxine is a known toxic component of snake venom. This may be seen both from its cytopathic effects on normal cells (pg. 5, lines 44-46), and also from its low LD_{50} which is in the range of its therapeutic dose (see document D2, pg. 1928, 1st full paragraph and pg. 1935, 2nd para., and excerpt from the Karlsson reference cited in D2 and enclosed herein [Annex I]).

6. In summary, it is believed that D1 neither teaches the invention nor renders it obvious.

The Examiner also cites document D2 as anticipating the novelty of the invention. Applicant respectfully disagrees for the following reasons:

1. The analgesic effect of crotamine as described in D2 is a morphine-like effect, i.e. it is short-acting and is administered *after* induction of pain to reduce acute pain effects. This may be seen from the analgesic activity assay (pg. 1929), in which the sample was injected after induction of pain, and from the results in Figs. 3 and 4 from which it may be seen that the analgesic effect declines after approximately one hour. The authors specifically compare crotamine to morphine (pg. 1933, 2nd para.).

2. The fraction of the invention, on the other hand acts after a lag period which may extend for one or more days. It is therefore more adapted for treatment of chronic pain (see page 6, lines 15-18, and page 7, lines 25-31). We enclose results of further experiments in which the fraction of the invention was administered up to 4-5 days before pain induction (Annex II). This is clearly not the crotamine protein whose effects begin to decline after one hour!

3. Furthermore, as pointed out in D2 (pg. 1935, 2nd paragraph), crotamine is a known myonecrotic which causes structural damage to skeletal muscle. We enclose the a reference by Cameron and Tu (Annex III) relating to this toxicity aspect of crotamine.

4. In summary, it is believed that D2 does not teach the invention.

If any issues remain outstanding, the Examiner is encouraged to contact Applicant's representative at the following number: 972-3-7109333.

Yours very truly,
REINHOLD COHN AND PARTNERS
By:

Jonathan Patinkin

JP/00030

CLAIMS:

1. A substantially non-toxic fraction isolated from snake venom having the characteristics of a fraction purified from said venom by Mono Q ion-exchange chromatography, wherein said fraction has an analgesic effect after a lag period.

2. A fraction according to Claim 1 wherein said chromotography is carried out on a Mono Q column in 20mM Tris-HCl buffer pH 7.0, and the fraction elutes at 12-28 minutes.

3. A fraction according to Claim 1 wherein said snake is selected from the group of snake families consisting of *Viperidae, Elapidae, Crotolidae, Hydrophidae and Atractaspidae*.

4. A fraction according to Claim 3 wherein said *Viperidae* is *Vipera xanthina*.

5. A fraction according to Claim 4 wherein said *Vipera xanthina* is *Vipera xanthina palestinae*.

6. A fraction according to Claim 3 wherein said *Crotolidae* is *Crotalus adamanteus*.

7. A fraction according to Claim 3 wherein said *Elapidae* is *Naja melanoleuca*.

8. A product obtained from the fraction of Claim 1 which retains said properties of the fraction.

9. A derivative of the product of Claim 8 which retains said properties of the fraction of Claim 1.

10. Use of a fraction according to Claim 1 in the preparation of a pharmaceutical composition for use as an analgesic acting after a lag period.

11. A pharmaceutical composition for use as an analgesic comprising a substantially non-toxic fraction according to Claim 1 and a pharmaceutically acceptable carrier or excipient.

12. A pharmaceutical composition according to Claim 11 for topical administration.

13. A pharmaceutical composition according to Claim 11 for parenteral administration.

CLAIMS:

1. A substantially non-toxic fraction isolated from snake venom having the characteristics of a fraction purified from said venom by Mono Q ion-exchange chromatography, wherein said fraction has an analgesic effect, after a lag period.

2. A fraction according to Claim 1 wherein said chromotography is carried out on a Mono Q column in 20mM Tris-HCl buffer pH 7.0, and the fraction elutes at 12-28 minutes.

3. A fraction according to Claim 1 wherein said snake is selected from the group of snake families consisting of *Viperidae, Elapidae, Crotolidae, Hydrophidae and Atractaspidae*.

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9. A derivative of the product of Claim 8 which retains said properties of the fraction of Claim 1.

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11. A pharmaceutical composition for use as an analgesic comprising a substantially non-toxic fraction according to Claim 1 and a pharmaceutically acceptable carrier or excipient.

12. A pharmaceutical composition according to Claim 11 for topical administration.

13. A pharmaceutical composition according to Claim 11 for parenteral administration.

ANNEX I

Snake Venoms

Contributors

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Editor

Chen-Yuan Lee

With 208 Figures

Springer-Verlag Berlin Heidelberg New York 1979

In: Snake Venoms ANNEX I
ed. Chen-Yuan Lee
Springer-Verlag, New York 1979)

When the three subunits combine the enzymatic activity of the α-subunit is suppressed and the lethality compared on a molar basis is increased about 500-fold (FOHLMAN et al., 1976). No explanation can be given yet for this phenomenon. Similar effects have been observed with crotoxin (Sect. C.III).

The γ-subunit is clearly homologous to phospholipases but has an extra N-terminal octapeptide which is homologous to the activation peptide of the porcine prophospholipase A_2 (Fig. 11).

III. Crotoxin

Crotoxin, the main neurotoxin of the South American rattlesnake *Crotalus durissus terrificus*, was isolated in a crystalline form as early as 1938 by SLOTTA and FRAENKEL-CONRAT. The original isolation method is still used. The venom is dissolved in dilute (0.013–0.016 M) HCl, heated for 10 min at 70° C, and the coagulated protein is centrifuged off. Crotoxin is then precipitated at its isoelectric point pH 4.7–4.8 (HENDON and FRAENKEL-CONRAT, 1976; BREITHAUPT, 1976a). The amorphous toxin can be crystallized from pyridine acetate at pH 4.4 upon slow cooling from 55° C (SLOTTA and FRAENKEL-CONRAT, 1938; SLOTTA, 1955).

Crystalline crotoxin was homogeneous in ultracentrifugation (GRALÉN and SVEDBERG, 1938) and in moving boundary electrophoresis (LI and FRAENKEL-CONRAT, 1942), but crotoxin obtained after isoelectric precipitation seems to be a non stoichiometric complex of several components, the proportions of which can also vary from one preparation to another (BREITHAUPT et al., 1975). This variation probably explains the varying lethal doses and some other divergent data shown in Table 2. The high toxicity is due to a complex between a basic phospholipase A, also called *crotoxin* B (basic), and an acidic protein, *crotoxin* A (acidic) or *crotapotin* (HENDON and FRAENKEL-CONRAT, 1971; RÜBSAMEN et al., 1971).

Crotoxin can be dissociated by ion exchangers. BREITHAUPT et al. (1975) adsorbed it to CM cellulose at pH 3.5 and separated the constituents by gradient chromatography. Two basic phospholipases accounted for 48% of the protein, two crotapotins accounted for 31% (the later eluting crotapotin peak might be a chromatographic artifact; see also RÜBSAMEN et al., 1971), and minor constituents (an acidic phospholipase A, crotamine, and other proteins) accounted for 8.4%. The recovery was 87.4%, which is normal in chromatography of proteins after adsorption to cellulose ion exchangers.

The basic phospholipase is moderately lethal (540 μg/kg mouse, Table 2) but upon recombination with crotapotin, practically full lethality can be restored (HENDON and FRAENKEL-CONRAT, 1971; RÜBSAMEN et al., 1971; BREITHAUPT et al., 1975). Both phospholipases isolated by BREITHAUPT et al. (1975) combine with crotapotin. The lethality of the basic phospholipase can also be potentiated by molecules other than crotoxin A or crotapotin. The nonlethal *volvatoxin* A2 (mol wt 25000, isoelectric point 4.5), a component of a mushroom cardiotoxin (LIN et al., 1973) forms a complex with the basic phospholipase and augments its toxicity significantly (JENG and FRAENKEL-CONRAT, 1976).

Crotapotin not only increases the toxicity of the basic phospholipase but also suppresses its enzyme activity by as much as 90% (BREITHAUPT, 1976a). A contrary result has, however, been obtained by FRAENKEL-CONRAT et al. (1976) who reported that crotoxin and crotoxin B have equal catalytic activities.

Table 2. Crotoxin and its subunits

	LD_{50} μg/kg mouse	Molecular weight	Isoelectric point
Crotoxin	i.p. 35[a] s.c. 370[d] i.p. 60[a] s.c. 500[e] i.v. 110[e]	30,000[c] 21,000[f] ~25,000[g]	4.7[k] 4.8–5.1[l]
Basic subunit phospholipase A (crotoxin B[a])	i.v. 540[e]	14,500[m] 15,800[n] 16,300[o] 13,000[b] 11,300[g]	9.5–9.8[l] 8.6[i]
Acidic subunit (crotoxin A[a] or crotapotin[c])	Nontoxic i.v. >50,000[e]	9,500[o] 8,400[b] 9,300[h]	3.4[l] 3.7[i]

[a] Hendon and Fraenkel-Conrat (1971), two preparations of toxin.
[b] Hendon and Fraenkel-Conrat (1971), amino acid composition.
[c] Reibsamen et al. (1971).
[d] Slotta and Fraenkel-Conrat (1938).
[e] Gralén and Svedberg (1938) ultracentrifugation.
[f] Horst et al. (1972) elutes in gel filtration slightly after chymotrypsinogen A (mol. wt. 25,200).
[g] Horst et al. (1972) gel filtration in 6 M GuHCl of reduced and alkylated molecule.
[h] Horst et al. (1972) gel filtration in 6 M GuHCl of unreduced molecule.
[i] Horst et al. (1972) isoelectric focusing.
[j] Rodríguez and Scannone (1976) gel filtration.
[k] Li and Fraenkel-Conrat (1942) moving boundary electrophoresis.
[l] Breithaupt et al. (1974) isoelectric focusing.
[m] Breithaupt et al. (1974) gel filtration in 6 M GuHCl of reduced and alkylated molecule.
[n] Breithaupt et al. (1974) ultracentrifugation and dodecylsulfate-gel electrophoresis
[o] Breithaupt et al. (1974) amino acid composition.

From the yields of the basic phospholipases (48%) and crotapotin(s) (31%) obtained by Breithaupt et al. (1975), and using the molecular weights determined in the same laboratory, the molar ratio phospholipase-crotapotin can be calculated. It varies between 0.90 and 1.02. The classic crotoxin thus appears to be a 1:1 complex of phospholipase-crotapotin. Since there are at least two homologues of the phospholipase, there should be at least two different neurotoxic complexes, coprecipitated with varying amounts of other proteins which do not contribute significantly to the toxicity.

Electrostatic interaction is probably decisive for holding crotoxin together. Like taipoxin it is dissociated by interaction with ion exchangers but can survive gel filtration (Hendon and Fraenkel-Conrat, 1976; Rodríguez and Scannone, 1976) and electrophoresis in a pH interval at least 4–7 (Li and Fraenkel-Conrat, 1942). The stability of the complex depends on the pH, i.e., the charge of the two constituents. At low or high pH values, when the two components have the same charge, the complex probably dissociates. A chromatographic isolation method based on a combination of gel filtration and electrophoresis analogous to that used for taipoxin might be an alternative to the procedure presently used, which historically belongs to the prechromatographic period of protein chemistry.

Crotoxin B has one histidine residue which reacts with p-bromophenacyl bromide. The modified enzyme is devoid of both lethality and catalytic activity. It can still combine with crotoxin A, but the complex is inactive. The crotoxin complex does not react with the reagent, nor does crotoxin A (FRAENKEL-CONRAT et al., 1976).

The two basic phospholipases isolated from the crotoxin complex by BREITHAUPT et al. (1975) have a phenylalanine residue at position 11, whereas the other phospholipases and toxin constituents have half-cystine (Fig. 11). The crotoxin components obviously have a different disulfide pairing, and they might, therefore, be homologous only to a limited extent with the other proteins.

Crotapotin consists of three peptide chains containing 40, 34, and 14 residues and joined by disulfide bonds (BREITHAUPT et al., 1974). This unusual structure might be the result of proteolytic fragmentation of a single-chain precursor molecule or an artifact due to hydrolysis of particularly susceptible peptide bonds, such as Asp-Pro bonds, during the heat coagulation of the venom at low pH.

IV. β-Bungarotoxin

The venom of *Bungarus multicinctus* was initially fractionated by zone electrophoresis in a starch block into three neurotoxic fractions called α-, β-, and γ-*bungarotoxin*. A postsynaptic type of neuromuscular blocking action was found in the α-fraction, whereas the action of the β- and γ-fractions appeared to be exclusively presynaptic (CHANG and LEE, 1963). Chromatography on CM-Sephadex C-50 resolved the venom into a great number of components and there seem to be at least six toxins with presynaptic activity (LEE et al., 1972; ĐERDJOVIĆ et al., 1975). The somewhat different chromatographic patterns obtained in the two laboratories may reflect differences between the venom samples. The LD_{50} (i.p.) varied greatly for the different toxins. The lowest were in the range 10–20, the intermediate ones about 50, and the highest ones about 300 up to as high as 2000 μg/kg mouse.

Since the venom contains a great number of components, gel filtration should be suitable for an initial fractionation. It should at least separate presynaptic toxins from the postsynaptic ones and facilitate further purification by ion exchangers.

One toxin, LD_{50} (i.p.) 14 μg/kg mouse (LEE et al., 1972), consists of two subunits of molecular weight 8800 and 12400 held together by disulfide bonds (KELLY and BROWN, 1974).

V. Enhydrina schistosa Myonecrotic Toxins

The postsynaptic neurotoxins account for about 60% of the protein of *Enhydrina schistosa* (common sea snake) venom (KARLSSON et al., 1972a); nevertheless the clinical picture of poisoning is myotoxic rather than neurotoxic. Muscle-movement pains and myoglobinuria are the most characteristic symptoms in human snake bite victims (REID, 1961).

A toxin which produces myoglobinuria in mice upon intravenous injection has recently been purified by gel filtration on Sephadex G-75 and ion-exchange chromatography on Bio-Rex 70 (FOHLMAN and EAKER, 1977). This toxin, like notexin, is preferentially freeze-dried from 0.01 M ammonium acetate. It is a basic protein containing 120 residues and seven disulfides and has a molecular weight of 13500.

At doses above 100 μg/kg, the mice die rather rapidly as a result of respiratory failure, the syndrome resembling that caused by the presynaptic toxins notexin and

Annex II

Experiment:

Analgesic assay was carried out as described on pages 7-8 of the application.

The ointment with (0.05 mg /g ointment) and without (control) the fraction of the invention was topically applied 6 times over a period of 4 weeks. The analgesic assay was applied 4-5 days after the last treatment.

Results:

	Mean no. of licks ± SE	**no. of hamsters**	**p**
Control	83.8 ± 11.8	21	
Vipera russelli Mono Q fraction	47.6 ± 7.7	20	0.02
Crotalus adamanteus Mono Q fraction	25.3 ± 5.3	20	0.00

Conclusions:

The analgesic effect of the fraction of the invention is observed at least 4-5 days after its application.

ANNEX III

Biochimica et Biophysica Acta, 532 (1978) 147—154
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BBA 37829

CHEMICAL AND FUNCTIONAL HOMOLOGY OF MYOTOXIN *a* FROM PRAIRIE RATTLESNAKE VENOM AND CROTAMINE FROM SOUTH AMERICAN RATTLESNAKE VENOM

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(Received July 8th, 1977)

Summary

Myonecrosis is a serious result of rattlesnake bite and constitutes a persistent clinical problem. In the current study we have isolated crotamine from the venom of *Crotalus durissus terrificus* to test its ability to cause structural damage to skeletal muscle, and to make direct chemical comparisons with Myotoxin *a*, a myotoxic polypeptide we recently isolated from prairie rattlesnake (*Crotalus viridis viridis*) venom. Disc gel electrophoresis, isoelectric focusing, circular dichroic spectroscopy, and amino acid analysis, all indicated a high degree of chemical similarity. Light microscope histology revealed that crotamine caused vacuolization of skeletal muscle fibers, qualitatively the same as the vacuolization caused by Myotoxin *a*. The ability of these two basic snake venom polypeptides to cause structural damage to skeletal muscle fibers has significant implications toward more complete understanding of the cause of snake venom-induced myonecrosis.

Introduction

Despite the toxicological and clinical significance of snake venom-induced myonecrosis, the phenomenon is relatively poorly understood. Electron microscopic examination of mouse skeletal muscle injected with crude prairie rattlesnake (*Crotalus viridis viridis*) venom revealed very severe and complex damage at the ultrastructural level [1]. Virtually every feature of the muscle fiber underwent structural change as a result of the crude venom action. To promote better understanding of the causes of structural change, individual myotoxic components of snake venoms must be isolated, and the chemical basis for their

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ability to damage muscle must be explained. In a prior study, we isolated a previously unknown polypeptide myotoxin, designated "Myotoxin *a*", from crude prairie rattlesnake venom [2]. The initial effect of Myotoxin *a* on skeletal muscle was shown to be vacuolization of muscle fibers. This effect was also the initial structural change observed with the electron microscope [3].

Crotamine is a polypeptide causing paralysis which was first isolated from *Crotalus durissus terrificus* (tropical rattlesnake) venom in South America [4]. Distinct from crotoxin in the same venom, crotamine was originally described as a neurotoxin [5], but is now known to cause depolarization of muscle cell membranes [6]. Because of similarities between Myotoxin *a* and the reported characteristics of crotamine, we performed direct chemical comparisons between Myotoxin *a* and crotamine. We also tested the ability of crotamine to cause structural damage to skeletal muscle fibers. Though the physiological effects of crotamine have been documented, no structural effects on muscle have been reported prior to the current study.

Materials and Methods

Myotoxin *a* was isolated from prairie rattlesnake venom purchased from Miamie Serpentarium Laboratories [2]. Crotamine was isolated from *Crotalus durissus terrificus* (tropical rattlesnake) venom purchased from Sigma Chemical Co.

Crude tropical rattlesnake venom was subjected to acetic acid extraction and ethanol fractionation as described by Giglio [7]. 100 mg of the crude venom was suspended in 6.0 ml of 0.05 M acetic acid at 0°C. A small insoluble residue was removed by centrifugation and discarded. The acetic acid extract was stirred in an ice bath, and 50% ethanol (v/v) at −20°C was slowly added to a final concentration of 20%. This was centrifuged at 4°C, and the pellet was discarded. The clear supernatant was dialyzed against distilled water, then lyophilized.

The lyophilisate was taken up in 0.05 M Tris buffer (pH 9.0) containing 0.1 M KCl, and was applied to a C-25 carboxymethyl Sephadex column (1 × 10 cm) equilibrated with the same buffer. The column was developed with a stepwise KCl salt gradient in the Tris buffer. Appropriate tubes were pooled, dialyzed in benzoylated tubing, and lyophilized.

Disc gel electrophoresis was done in the β-alanine system [8]. Isoelectric focusing was done in 7.5% polyacrylamide gels containing 2% pH 3.5-10 LKB ampholytes. The upper reservoir contained 0.01 M boric acid (pH 5.5) and the lower contained 0.05 M piperdine (pH 11.8).

Myotoxicity was evaluated by light microscope histology by injecting 50-μg samples of protein in 0.1 ml of 0.9% NaCl into the medial aspect of the thigh muscles of Swiss Webster white mice. Mice were killed 72 h after injection. Tissue samples were removed from the back of the thigh, fixed for 16 h in Bouin's fixative [9], washed, dehydrated with ethanol, and embedded in paraffin. Sections were stained with hematoxylin and eosin for examination with the light microscope.

Amino acid analysis was done with a JEOL Model JLC-GAH analyzer. Cir-

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December 17, 2001

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OF 2 PAGE(S)
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SWITZERLAND

Dear Sirs,

Re: PCT Application No. PCT/IL99/00386
"Analgesic From Snake Venom"
Shulov Institute for Venom Research Ltd.
Int. Filing Date: 14.07.1999
Our Ref: 117025-7 DB

We herewith **request the IB to record the change of name** of the applicant company as follows:

S.I.S. Shulov Institute for Science Ltd.

Enclosed herewith is a corrected page 1 of the Request.

In view of the close deadline for entering National Phase, we would very much appreciate your faxing us a copy of the Form PCT/IB/306 as soon as possible (in any case before the end of the month).

Thank you very much in advance for your assistance.

Yours very truly,
REINHOLD COHN AND PARTNERS
By:

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PCT REQUEST

117025.7 DB

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0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	

0-4	**Form - PCT/RO/101 PCT Request**	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.03.2001)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Israel Patent Office (RO/IL)
0-7	**Applicant's or agent's file reference**	117025.7 DB
I	**Title of invention**	ANALGESIC FROM SNAKE VENOM
II	**Applicant**	
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REPLY UNDER RULE 66.3

Attention: Ms. Eva Bohmerova

Dear Sirs,

Re: Patent Application No. PCT/IL99/00386
"Analgesic from snake venom"
in the name of S.I.S Shulov Institute For Science Ltd.
Inventors: PRIMOR Naftali and SHULOV Aharon
Our Ref: 117025-7

This is in response to the Written Opinion of July 23, 2001, in accordance with PCT Rule 66.3. We enclose with the courier copy of this letter amended pages 18 and 19 in triplicate, and the original pages in which the amendments have been indicated.

Original claims 1 and 17 have been amended by defining the snake families, and expressly excluding snakes of the species *Vipera xanthina*. Original claims 3-5 have been cancelled, and the subsequent claims renumbered accordingly. Support for this amendment appears on page 5, lines 21-23, and in original Claim 3. This amendment overcomes the objection under Art. 33(2) with respect to reference D3.

As in our previous response, original Claims 1 and 10 have been amended by defining the analgesic effect as occuring after a lag period. We believe that this lag period is defined in the specification as extending for at least one day before induction of pain (see 4th paragraph of page 1 of our letter of June 24, 2001). This lag period is not typical of analgesics in general, e.g. morphine-like analgesics which are short-acting (see our comments regarding D2 in our afore mentioned letter).

With respect to the objection of lack of inventive step under Art. 33(3), we believe that the presence of an analgesic substance in the venom of one species of snake does not render obvious that other or all species of snakes will also have

such a substance in their venom. The Examiner's attention is drawn to page 2 of the specification, the 3rd paragraph, which states:

> *Snake venom comprises a large variety of different substances. Out of several hundreds of estimated compounds, it is believed that only 4-8 are involved in the toxic effect of the venom. Despite functional similarity, snake venoms differ considerably in their chemical composition. Each species possesses it's own characteristic venom composition. To date, only a few hundred compounds from some 400 venomous snake species have been characterized. These include enzymes, toxins, growth factors, etc. Most of the isolated venom compounds are of unknown function.*

Thus, the venom of each snake species is unique, and finding a substance in the venom of one has no bearing on whether the same substance will be present in the venom of another. The present invention concerns the surprising and unexpected finding that all of the claimed snake families have the same analgesic substance. Although the analgesic effect of snake venom may have been previously known, the specific fraction having the specific characteristics as defined in Claim 1 was unknown and unobvious.

In summary, we believe that the claims are both novel and inventive over the prior art.

Yours very truly,
REINHOLD COHN AND PARTNERS
By:

Jonathan Patinkin

JP/00035

CLAIMS:

1. A substantially non-toxic fraction isolated from snake venom having the characteristics of a fraction purified from said venom by Mono Q ion-exchange chromatography, wherein said fraction has an analgesic effect after a lag period, and wherein said snake is selected from the group of snake families consisting of *Atractaspidae, Elapidae, Crotolidae, Hydrophidae* and *Viperidae*, with the exception of *Vipera xanthina.*

2. A fraction according to Claim 1 wherein said chromotography is carried out on a Mono Q column in 20mM Tris-HCl buffer pH 7.0, and the fraction elutes at 12-28 minutes.

3. A fraction according to Claim 1 wherein said *Crotolidae* is *Crotalus adamanteus*.

4. A fraction according to Claim 1 wherein said *Elapidae* is *Naja melanoleuca*.

5. A product obtained from the fraction of Claim 1 which retains said properties of the fraction.

6. A derivative of the product of Claim 5 which retains said properties of the fraction of Claim 1.

7. Use of a fraction according to Claim 1 in the preparation of a pharmaceutical composition for use as an analgesic acting after a lag period.

8. A pharmaceutical composition for use as an analgesic comprising a substantially non-toxic fraction according to Claim 1 and a pharmaceutically acceptable carrier or excipient.

9. A pharmaceutical composition according to Claim 8 for topical administration.

10. A pharmaceutical composition according to Claim 8 for parenteral administration.

11. A pharmaceutical composition according to Claim 8 for the treatment of pain.

12. A method for the relief of pain of a subject comprising administrating to said subject a substantially non-toxic fraction according to Claim 1.

13. A method according to Claim 12 wherein said fraction is topically administered.

14. A method for isolating a substantially non-toxic fraction from snake venom, wherein said fraction has an analgesic effect, comprising applying whole venom to an ion exchange column and eluting the fraction with an aqueous buffer, wherein said snake is selected from the group of snake families consisting of *Atractaspidae, Elapidae, Crotolidae, Hydrophidae* and *Viperidae*, with the exception of *Vipera xanthina*.

15. A method according to Claim 14 wherein said column is a Mono Q ion-exchange column.

16. A method according to Claim 15 wherein said column is eluted with a Tris-HCl buffer or with an ammonium acetate buffer.

17. A method according to Claim 16 wherein the concentration of said buffer is 20mM and the pH is in the range of 6.8-7.5.

18. A method according to Claim 17 wherein said fraction elutes at 12-28 minutes.

CLAIMS:

~~1.~~ A substantially non-toxic fraction isolated from snake venom having the characteristics of a fraction purified from said venom by Mono Q ion-exchange chromatography, wherein said fraction has an analgesic effect after a lag period, and wherein said snake is selected from the group of snake families consisting of *Atractaspidae, Elapidae, Crotolidae, Hydrophidae* and *Viperidae*, with the exception of *Vipera xanthina*.

~~2.~~ A fraction according to Claim 1 wherein said chromotography is carried out on a Mono Q column in 20mM Tris-HCl buffer pH 7.0, and the fraction elutes at 12-28 minutes.

~~3. A fraction according to Claim 1 wherein said snake is selected from the group of snake families consisting of *Viperidae, Elapidae, Crotolidae, Hydrophidae and Atractaspidae.*~~

~~4. A fraction according to Claim 3 wherein said *Viperidae* is *Vipera xanthina*.~~

~~5. A fraction according to Claim 4 wherein said *Vipera xanthina* is *Vipera xanthina palestinae*.~~

~~6.~~ [3.] A fraction according to Claim ~~3~~ 1 wherein said *Crotolidae* is *Crotalus adamanteus*.

~~7.~~ [4.] A fraction according to Claim ~~3~~ 1 wherein said *Elapidae* is *Naja melanoleuca*.

~~8.~~ A product obtained from the fraction of Claim 1 which retains said properties of the fraction.

~~9.~~ [6.] A derivative of the product of Claim ~~8~~ 5 which retains said properties of the fraction of Claim 1.

~~10.~~ [7.] Use of a fraction according to Claim 1 in the preparation of a pharmaceutical composition for use as an analgesic acting after a lag period.

~~11.~~ [8.] A pharmaceutical composition for use as an analgesic comprising a substantially non-toxic fraction according to Claim 1 and a pharmaceutically acceptable carrier or excipient.

~~12.~~ [9.] A pharmaceutical composition according to Claim ~~11~~ 8 for topical administration.

~~13.~~ [10.] A pharmaceutical composition according to Claim ~~11~~ 8 for parenteral administration.

~~14.~~ [11.] A pharmaceutical composition according to Claim ~~11~~ 8 for the treatment of pain.

~~15.~~ [12.] A method for the relief of pain of a subject comprising administrating to said subject a substantially non-toxic fraction according to Claim 1.

~~16.~~ [13.] A method according to Claim ~~15~~ 12 wherein said fraction is topically administered.

~~17.~~ [14.] A method for isolating a substantially non-toxic fraction from snake venom, wherein said fraction has an analgesic effect, comprising applying whole venom to an ion exchange column and eluting the fraction with an aqueous buffer, wherein said snake is selected from the group of snake families consisting of *Atractaspidae, Elapidae, Crotolidae, Hydrophidae* and *Viperidae*, with the exception of *Vipera xanthina*.

~~18.~~ [15.] A method according to Claim ~~17~~ 14 wherein said column is a Mono Q ion-exchange column.

~~19.~~ [16.] A method according to Claim ~~18~~ 15 wherein said column is eluted with a Tris-HCl buffer or with an ammonium acetate buffer.

~~20.~~ [17.] A method according to Claim ~~19~~ 16 wherein the concentration of said buffer is 20mM and the pH is in the range of 6.8-7.5.

~~21.~~ [18.] A method according to Claim ~~20~~ 17 wherein said fraction elutes at 12-28 minutes.